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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Art Unit: 1805
CLASSEN, J.B.	Examiner: N. Vogel
Serial No.:08/104,529	Washington, D.C.
Filed: August 12, 1993	July 8, 1994
For: METHOD AND COMPOSITION FOR AN EARLY VACCINE TO PROTECT...	Docket No.: CLASSEN=1

DECLARATION OF JOHN B. CLASSEN

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

S i r :

1. I am a medical doctor and the sole inventor named on the present application.

2. I have conducted an experiment to demonstrate that the method of the present invention is effective in an animal model other than NOD mice.

Diabetic prone BB rats were immunized according to the method disclosed in the specification in order to show that the method of immunization could prevent diabetes in other species beside NOD mice. BB rats spontaneously develop diabetes at an early age as is the case in NOD mice and humans. Many of the findings present in human type I diabetics and in NOD mice are found in BB rats leading experts to believe diabetes in BB rats is also a autoimmune disorder. Insulitis develops in the pancreas of BB rats before the onset of diabetes while antibodies develop to islet cells and possibly to insulin. Diabetes can be prevented by neonatal thymectomy as well as administration during the prediabetic period of cyclosporine, anti-lymphocyte antibodies, or purified lumphokines like TNF. Genetic experiments show that diabetes is closely linked to the MHC class II genes in BB rat as it is in humans. Many older rats develop autoimmune thyroiditis that is usually related to the development of diabetes as occurs in humans.

BB rats have an immunologically distinct disease from the disease in NOD mice. Diabetes develops in approximately equal numbers of males and females in contrast to NOD mice where disease develops more commonly in females. The incidence of diabetes in BB rats is not affected by gonadectomy or the administration of androgens as occurs with NOD mice. In contrast to humans and NOD mice, BB/Wor rats, the most commonly used substrain of BB rats, are severely lymphopenic. They have a marked decreased number of mature T lymphocytes in peripheral blood, spleen and lymph nodes. The CD4+ subset is substantially reduced but the CD8+ subset is almost completely absent. Natural killer cells are relatively over expressed. Several review papers have been published on this model Modes, et al, 1987; Parfrey, et al, 1989; Crisa et al., 1992.

BB rats were immunized with a combination of the anthrax and DTP vaccines (n=20) or nothing as a control (n=28). Groups contained approximately equal number of male and female rats. The vaccinated group was given the following dosing schedule: day 1 (.1ml, 1:5); day 4 (.15ml, 1:5), day 11 (.15ml, 1:5), day 25 (.2ml, 1:5), day 39 (.2ml, 1:5), day 53 (.2ml, 1:5) day 61 (.2ml,1:2.5), and every 14 days for 3 more injections at approximately (.2ml, 1:2.5). Days of injection varied by one at times. The notation 1:5 means 1 part vaccine to 5 parts PBS. At 16 weeks of age 54% of the untreated rats had developed diabetes and/or died compared to 20% in the vaccinated group. At 20 weeks of age 54% of the untreated rats had developed diabetes and/or died compared to 25% in the vaccinated group. At 32 weeks the results were 54% versus 35% respectively (graph 1) which represents a 34% reduction in the incidence of diabetes. The difference between the two groups were statistically significant at 20 weeks (P=0.027). The findings that the method of immunization can prevent diabetes in both NOD mice and BB rats provides strong proof that methods of immunization presented in the specification have

the ability to prevent chronic immune mediated diseases in mammals with very different genetic defects.

3. While its significance have not hitherto been recognized, epidemiology data assembled and correlated in example 4 of the specification (page 82 Line 17- page 84 Line 21) shows that diabetes mellitus in humans, as with NOD mice and BB rats, may be inhibited or induced by the administration of human vaccines. Epidemiology data is included below to provide further proof that diabetes in humans may be enhanced or prevented by the administration of standard doses of pediatric vaccines. The data includes correlations between the specific immunization schedules and the incidence of diabetes in western European countries as well as temporal studies showing changes in the incidence of diabetes in countries after the immunization schedules were changed. Additional epidemiology data is provided to explain the mechanism of action of this phenomenon and give further support for the data showing vaccination can prevent the development of immune mediated diabetes in humans.

3.1. Intercountry analysis

An epidemiology study described in example 4 of the specification (page 82 line 17-page 84 line 21) showed that the incidence of diabetes in western European countries was closely correlated with a country's vaccination schedule. Western Europe was chosen because in a relatively small geographic area there are many different countries that have different immunization schedules, and the incidence of diabetes in these countries is known. The people in the western European countries have closely related racial backgrounds, diets, economic standards of living, and standards of health care. Eastern European countries of the former communist block were excluded because their standard of living and standard of medical care is not up to western levels.

The data correlating the incidence of diabetes to immunization schedule in western European countries is presented in table I. Several additional countries are included in table I that were not included in example 4 of the specification because the new data was collected after the patent was applied for. The additional countries include Portugal, Belgium, Luxembourg,and Malta. Several different regions in Italy, Spain and the United Kingdom are also included. References pertaining to the immunization schedules and incidence of diabetes of each country are included with table I. Note that the incidence of diabetes in Malta was significantly understated, according to the Schranz and Prikatsky, (Diabetic Medicine 6:228-231,1989) . The authors calculated the incidence of diabetes in Malta using the number of type I diabetics in public health clinics and the number of children of a particular age in the country. The authors admit significant number of diabetic children were treated in private clinics that were not accounted for.

The data in table I substantiates the previous findings presented in example 4 (page 82) of the specification. Administration of vaccines after 2 months increases the incidence of diabetes while administration of vaccines at birth can prevent diabetes. The findings are highly statistically significant. Administration of the pertussis vaccine after 2 months of age explains the higher incidence of diabetes in group 3 compared to most regions in group 1. Administration of the BCG vaccine after 2 months of age explains the higher incidence of diabetes in group 4 compared to group 3. Administration of the Hemophilus influenza vaccine after 2 months of age explains the higher incidence of diabetes in group 5 compared to 4. The ability of the BCG vaccine to protect against diabetes when administered at birth explains the lower incidence of diabetes in group 2 compared to group 3. There appears to be a correlation between immunization rate and the incidence of diabetes within some

groups since the districts in England and Ireland which had low immunization coverage with the pertussis vaccines compared to other countries in their groups (World Health Statistics Annual, 1991 page 62) had the lowest incidence of diabetes in their groups. Luxembourg had the highest incidence of diabetes in group 3 which can be explained by a significant number of individuals receiving the BCG vaccine even though it is not required (ibid). Minor variations within a group can be explained by genetic variation, environmental differences including epidemics, use of additional vaccines and the per cent of children fully immunized.

The incidence of diabetes in Sardinia is the one major exception to the above-stated patttern. Sardinia has a very high incidence of diabetes that cannot be explained by the routine pediatric immunization practices used on the island. The high incidence of diabetes can be explained, however, at least in part, by the extremely high incidence of thalassemia on the island.

3.2. Intracountry Temporal Analysis: Induction of Diabetes

Animal and human epidemiology data indicates that the incidence of diabetes will change when a country changes its immunization schedule. The data below suggests that if a vaccine is initially given after 2 months of age then the incidence of diabetes will go up. This situation was seen in both epidemiology studies of diabetes in Finland and the United States.

A.Finland

In Finland the incidence of diabetes had been very stable in the 0-4 year old age group from 1966-1975 until after the government made several changes to its vaccination schedule which was followed by large increases in the incidence of diabetes in the years 1976-1978 and 1988-1990 (Diabetologia 36:1303-1308, 1993). The rises in the incidence of diabetes can be explained by the addition of 3 new vaccines to the

Finnish vaccine schedule. A large clinical trial immunizing 130,000 infants aged 3 months to 5 years old with Hemophilus influenza or meningococci polysaccharide vaccines was started in November, 1974 (New England Journal Medicine 297:686-691, 1977). Finland has a small population with less than 65,000 children born each year (World Health Statistics Annul) so 130,000 represented a significant proportion of children in the country 0-4 years of age. In 1976 the pertussis vaccine in Finland was changed by the addition of a second strain of bacteria making the vaccine more antigenic (Acta Paediatric Scand 298 Suppl 21-25, 1982). The vaccine regiment was next altered to include the measles, mumps, rubella, (MMR) vaccine in 1982. A trial of Hemophilus influenza conjugate vaccine was initiated in January 1986 and included 114,000 children born between October 1,1985 and August 31,1987. Based on the results of the trial the Hemophilus influenza vaccine became part of the standard vaccine schedule in Finland starting in January of 1988 (New England Journal Medicine 317:1381-1387, 1990).

The changes in the incidence of diabetes especially in the 0-4 year old age group in Finland correspond temporally very closely with the changes in the vaccine schedule, supporting the rodent and the intercountry epidemiology data that the development of immune mediated diabetes is influenced by vaccination (table II). The use of the Hemophilus influenza vaccine as well as the addition of a second strain of bacteria to the pertussis vaccine explains the rapid rise of diabetes seen in the years 1977-1979 in 0-4 year olds . The discontinuation of the Hemophilus influenza vaccine can explain the drop in the incidence of diabetes in 0-4 year olds from 19.3 in the 1977-1979 cohort to 16.0 in the 1980-1982 cohort (Diabetes Care 8, Suppl 1:10-16, 1985). The widespread use of the MMR vaccine starting in 1982 and the Hemophilus influenza vaccine in 1986 can explain the large rise in the

incidence of diabetes in the 0-4 age group between the years 1980-1982 and 1987-1989 (Diabetologia 36:1303-1308, 1993).

Temporal data comparing the incidence of diabetes in different age groups suggest that the use of more antigenic vaccines schedules is causing the increased incidence of diabetes in Finland since the increased incidence is only seen in those who received the new vaccines. The temporal rises in the incidence of diabetes in the 0-4 year old age group occur shortly after the new vaccines are added but rises in the incidence of diabetes in the older age groups are delayed until those children immunized with the new vaccine reach the older age group. For example, following the use of the Hemophilus influenza/meningococcus vaccine in 1974 and new pertussis vaccine in 1976 the incidence of diabetes in the 1977-1979 cohorts increased compared to 1970-1976 cohorts by 63.5% in 0-4 year olds, 16% 5-9 year olds, 0% in 10-14 year olds (table II). Changes in incidence of diabetes were statistically more significant in the 0-4 age group, the age group that received the vaccine. When the incidence of diabetes started to decline in 0-4 year olds in 1980-1982 following discontinuation of the Hemophilus influenza/meningococci vaccine, the incidence continued to rise in 5-9 and 10-14 year olds as those who received the Hemophilus influenza/meningococci vaccine and the new pertussis vaccine entered these age groups.

B. Allegheny County,Pennsylvania (Greater Pittsburgh area)

Changes in the incidence of diabetes in Allegheny County,PA (Diabetes Care 16:1606-1611 1993) can also be explained by changes in immunization schedules, supporting findings discussed earlier that the incidence of immune mediated diabetes in humans is affected by vaccination. The epidemic of diabetes occurring in the 0-4 age group during the years 1985-1989, (table III) can be explained by the addition of the Hemophilus influenza vaccine to the immunization schedule. The FDA approved the Hemophilus influenza

polysaccharide vaccine in 1985 and the conjugated vaccine in 1987 (Jama 269:221-226, 1993). The vaccine was widely administered to children in Allegheny county. A study of its efficacy performed in Allegheny county showed that about 36% of children, chosen as controls, were immunized with the vaccine between August of 1985 and July of 1987 (Jama 260:1419-1422, 1988). Based on epidemiology and animal data discussed earlier it was predicted that the incidence of diabetes would rise in Allegheny County after wide spread use of the Hemophilus influenza vaccine occurred.

The drop in the incidence of diabetes in the 0-4 age group in years 1975-1979 can be explained by a drop in pertussis immunization. In September of 1974, at a time when there was international concern about the incidence of encephalitis caused by the pertussis vaccine, the Pennsylvania State government passed Act 210 of 1974 requiring children to receive certain immunizations prior to entering school (Pennsylvania Medicine December 1974, p41-42). The law required immunization with polio, diphtheria, tetanus, measles and rubella but not with pertussis vaccine. The law was a clear message that immunization with the pertussis vaccine was not considered necessary. Following the passage of this law incidence of diabetes in the 0-4 age group dropped about 61% in the 1975-1979 time period as compared to 1965-1969 ($P<.001$) as shown in table III. During the period of 1975 to 1979 immunization with the pertussis vaccine dropped of in several countries. Immunization with the pertussis vaccine was stopped in Sweden and pertussis vaccine acceptance in the United Kingdom fell from 75% in 1974 to 30% in 1978 (Pediatric Infectious Disease Journal 6:364-371, 1987).

The incidence of diabetes in the 0-4 age group during the period 1980-1984 rose to levels even higher than before 1975. This can be explained by the Pennsylvania Department of Health calling for increased immunization with the pertussis vaccine following an epidemic of pertussis in Pennsylvania in 1982

(Pennsylvania Medicine, March 1983, p16) and new state laws requiring that children receive the mumps vaccine (Pennsylvania Medicine, January 1983, p12-16). The results are consistent with previous findings that changes in immunization policy can lead to changes in the incidence of immune mediated diabetes.

3.3. Intracountry Temporal Analysis; Prevention of Diabetes

Epidemiology data from the Netherlands provides evidence that immunization with the smallpox vaccine at birth can prevent the development of diabetes. This data supports the intercountry epidemiology data that immunization at birth with the BCG vaccine can prevent diabetes. The findings also support the temporal data presented above that changes in immunization practices can effect the development of immune mediated diabetes in humans.

Epidemiology data shows that in the cumulative incidence of diabetes up to the age of 18 differed significantly in Danish birth cohorts (Diabetologia 35:139-142, 1992). There were two significant drops in the incidence of diabetes, one was centered around 1962 when the cumulative incidence dropped to 1.1 per 1000 ($P<.05$), and the other was centered around 1966 when the cumulative incidence dropped to 1.71 per 1000. The drops are in contrast to a cumulative incidence of diabetes outside of these troughs of about 1.98 per 1000 (table IV). The drops in 1962 and 1966 both occurred during smallpox epidemics in Europe and can best be explained by immunization of newborn infants in these periods with smallpox vaccines.

Western Europe had major epidemics of smallpox centered around the years 1962 and 1966. The epidemic in 1962 actually started in 1961 continuing into 1963 and included 158 cases from 7 western European countries. The 1966 epidemic included 72 cases and was limited to the United Kingdom. There was a strong emphasis placed on vaccination in smallpox epidemic of

1961-1963 as demonstrated by World Health Organization statistics showing 23.5 million Europeans were vaccinated with the smallpox vaccine in 1962 compared to an norm of about 11 million in nonepidemic years (International Symposium on Vaccination against communicable diseases Monaco 1973; Symp. Series Immunobiol. Standard 22:13-24.) Changes in smallpox vaccine acceptance were detected around 1962 and 1966 in the Netherlands (ibid pp271-276).

Literature from the 1960s and earlier suggested humans could be immunized at any time after birth, but immunization before 3 months of age was associated with an increased risk of encephalitis. It was customary at the time for physicians practicing in areas with a low incidence of smallpox to wait until the patient was several months old before administering the smallpox vaccine, however in areas with high incidence of smallpox, like third world countries, smallpox vaccine was often given at birth (International Symposium on Smallpox Vaccine, Bilthoven 1972. Symp. Series Immunobiol. Standard 19:243-248; Tubercle 43:155-160, 1962).

The common practice in the Netherlands in the 1960s was to immunize children with the smallpox vaccine starting at 2 months of age in normal, nonepidemic conditions (International Symposium on Smallpox Vaccine, Bilthoven 1972. Symp. Series Immunobiol. Standard 19:235-242). Given the fact that the literature recommends immunization earlier than usual in times of epidemics, it would have been expected that a number of physicians would have given the vaccine several weeks earlier as in 4 weeks of age or at birth. The resulting switch in immunization explains the drop in the incidence of diabetes in the cohorts born during smallpox epidemics.

3.4. Related epidemiology data

Epidemiology data suggests that diabetes in humans can be caused by transient immunological changes at birth which gives further support to the finding that immunization can

alter the development of diabetes in humans. A report has been published that maternal-child blood group incompatibility is associated with an increased risk for developing diabetes (OR =1.61) (Diabetologia 1992; 35:671-675). The more severe cases of the disorder had a higher incidence of the disease. Maternal-child blood group incompatibility has been shown by others (New England Journal of Medicine August 22, 1974; p.420) to cause transient suppression of the thymus/T cell immune system. These findings demonstrate that in humans, as in rodents, transient alterations to the developing immune system, immunosuppression or stimulation, will alter the risk of developing immune mediated diabetes or another chronic immune mediated disease later in life, enhance or decrease respectively.

3.5 Even though data on the incidence of diabetes has been collected for many years, in many countries, the art has not thought to correlate that incidence with childhood immunization schedules, and therefore did not recognize that the immunization methods of the present invention could be used to reduce the risk of developing immune mediated diabetes.

4. I have also performed a study to show that methods of immunization taught in the specification would also prevent spontaneous autoimmunity in MRL/lpr mice, which develop a disease that closely resembles Systemic Lupus Erythematosus or SLE in humans. The MRL/lpr mice, as with human SLE patients develop anti-DNA and anti-nuclear autoantibodies which can cause immune complexes. The immune complexes can cause causing arthritis, dermatitis, and fatal immune complex glomerulonephritis if untreated (J.Invest Dermatol 94:52-57, 1990). The data below proves that the methods taught in the patent application can prevent (or inhibit) two quite different autoimmune diseases, and, in my opinion, supports the inference that many other immune-mediated disorders can be similarly prevented or otherwise inhibited.

Pregnant MRL/MpJ-lpr mice (Jackson Laboratory, Bar Harbor, Maine) gave birth to pups which were injected with PBS or a combination of the anthrax vaccine and the acellular DTP vaccine. A series of nine intraperitoneal injections of the vaccines, diluted in PBS, were given to newborn mice using the following protocol: day 1 (.1 ml, 1:100), day 3 (.1 ml, 1:100), day 10 (.15 ml, 1:100 ml), week 4 and every 2 weeks through week 14 (.2 ml, 1:50). The notation 1:100 refers to one part vaccine to one part PBS. Weaning was done at approximately 21 days and only the female mice were used in the experiment. One group of 18 control mice received a similar injection schedule of PBS starting on day 1 and a second group of 20 control mice received a similar schedule (ie every 2 weeks through week 14) but starting at 4 weeks of age.

Urine was screened for the presence of protein, a common clinical test for glomerulonephritis. A few drops of mouse urine were placed on a urine protein dipstick (Albustix, Miles Inc, Elkhart, IN). At 13 weeks 6/38 (15.8%)of PBS control mice had a urine with a protein over 300mg/dl while 0/28 of the vaccinated mice had a urine with a protein over 300mg/dl. At 14 weeks 8/38 (21%) of PBS control mice had a urine with protein of over 300mg/dl while 0/27 of the vaccinated mice had developed urines with similar protein levels. At 15 weeks 10/38 (26.3%) of PBS control mice had a urine with protein of over 300mg/dl while 2/26 (7.7%) of the vaccinated mice had developed urines with similar protein levels. The results prove that the vaccination methods in the patent application are useful in the prevention of SLE.

The MRL data is important not only because it is a good model for human SLE but because this autoimmune disease is both genetically, immunologically, and clinically very different from diabetes.

5. Extensive animal experiments and epidemiology studies performed by the declarant have shown that a broad range of

human vaccines can influence the incidence of diabetes. The results are summarized in table VI below. The best if not only explanation for the ability of such a diverse group of agents to have an effect is through a common immunological pathway like release of certain lymphokines as described in the specification (page 12 line 7). Given the following reasons it is clear that one skilled in the art would predict that vaccines, not specifically tested, could be used to influence the development of diabetes in mammals.

The unconjugated Hemophilus influenza vaccine is a carbohydrate vaccine and it has been shown through epidemiology to alter the development of diabetes (para. 4). The DT vaccine contains protein antigens and has been shown to improve the anthrax-induced inhibition of diabetes development in diabetes prone rodents.

The broad variety of vaccines that alter the development of immune mediated diabetes suggests that the mechanism of action involves a general pathway, like release of lymphokines, as discussed in the specification (page 12 line 7). Purified lymphokines have been shown to alter the development of diabetes as discussed in the specification (page 10 line 7). Given that the data strongly supports that human vaccines at pharmaceutical doses are altering the development of diabetes through a general pathway not related to antigenic mimicry it would be highly unlikely that other human vaccines would not have the same effect. As added proof of our case epidemiology data performed after the filing showed that the Hemophilus influenza, smallpox, and the MMR vaccine alter the development of diabetes in humans.

6. Based on the information furnished in the specification, in conjunction with the general knowledge of the art, a person of ordinary skill can readily devise a suitable immunization schedule.

With regard to the timing of the initial dose, the data clearly shows that administration of vaccines starting after

2 months induces diabetes in mammals. Animal data supporting this conclusion include data from NOD mice where the pertussis vaccine is administered starting at 8 weeks example 2 (80 line 6-12). Epidemiology data supporting this include administration several vaccines starting after 2 months of age including the BCG vaccine (page 84 line 2), pertussis vaccine (page 83 line 26), Hemophilus influenza vaccine, and MMR. The time spans that mammals are vulnerable to vaccine induced diabetes extends for many years as demonstrated by epidemiology data that administration of the BCG vaccine to school age children is associated with increased incidence of diabetes (page 84 line 2).

On the other hand, starting immunization shortly after birth prevent both chronic immune mediated disorders, including diabetes, and infectious diseases. Data supporting the concept that immunization prior to 42 days allows some, though not optimal, protection against infectious diseases includes references on the DTP vaccine (page 3 lines 11-19), polio vaccine (page 5 line 20), hepatitis B vaccine (page 6 line 5), hemophilus influenza vaccine (page 5 line 27), and BCG vaccine (page 6 line 1). Data from mice showing that immunization with several different vaccines starting soon after birth can prevent the development of chronic immune mediated diseases are shown in example 1 (page 78 line 1), example 2 (page 79 line 1), and example 3 (page 81 line 1). This data has been confirmed in BB rats and in human epidemiology studies of the BCG vaccine (page 83 line 29) and the smallpox vaccine.

The specification provides ample information to predict how many administrations are necessary for a vaccine schedule to effectively prevent an chronic immune mediated disorder. Data from example 2 (page 79 line 1) showed that 9 injections greatly reduced diabetes in NOD mice compared to 3 injections in example 1. Epidemiology data in example 4 (page 83 line 29) showed that a single administration of BCG vaccine at birth

was able to reduce the incidence of diabetes but not abolish it.

Thus, the ability of an immunization schedule to inhibit diabetes is dependent on both amount of the vaccine given and how soon it is administered after birth. One skilled in the art would predict that the more frequently an vaccine is administered the more effective it is at inhibiting immune mediated diseases like diabetes, especially when higher doses are administered. Data in example 2 (page 79) show that a frequency of administration of approximately every 2 weeks is sufficient to completely prevent diabetes in NOD mice.

Extensive coverage of screening methods (page 56 line 27) was included in the specification to allow one skilled in the art to create his or her unique immunization schedule without undue experimentation. One skilled in the art can use the screening methods to alter the immunization schedule recommended in the preferred embodiment. Example 2 (page 79) shows that an experiment with as few as 25 mice can be used to experimentally confirm the predictions of one skilled in the art pertaining to a new immunization schedule he or she may want to develop. The value of the NOD mice data is reinforced by the fact that BB rats and humans (page 82) respond similarly to NOD mice.

7.0 There is a minimum dose of a particular vaccine needed to induce protection against infectious diseases. These doses are mentioned in the specification in multiple places (page 34 line 18; page 35 line 4; page 26 line 15-page 27 line 10). Those skilled in the art are aware that additional information on a particular vaccine is available from the FDA through the freedom of information act(page 26 line 29), patent literature (page 26 line 27), published results of clinical trials, the United States Pharmacopedia,the code of federal regulations and package inserts that come with all FDA regulated pharmaceuticals, including vaccines.

Doses of vaccines used to prevent infectious diseases have been shown to prevent chronic immune mediated disease such that one skilled in the art can predict what amounts of immunogens are needed to yield results similar to those in examples 1-3 (page 78-82) of the specification. For example, epidemiology data has clearly shown (page 82) that the incidence of diabetes is profoundly affected by standard pediatric doses of childhood vaccines. Animal data in examples 1 (page 78), 2 (page 79) and 3 (page 81) have shown that chronic immune mediated disorders in animals are also prevented using doses that would be acceptable to human use. One skilled in the art would readily realize that one could use higher doses as long as toxicity was minimal but using lower doses would decrease the ability to prevent infectious diseases.

Screening methods (page 56 line 27) were included in the specification to allow one skilled in the art to develop a new immunization schedule using doses different from standard pediatric doses without undo experimentation. Example 2 (page 79) shows that an experiment with as few as 25 or even less mice can be used determine if the new dose is adequate. The value of the NOD mice data is reinforced by the fact that BB rats and humans (page 82) respond similarly to NOD mice.

8. Many chronic immune mediated diseases share common immunological pathways. Evidence supporting this has been included in the specification (page 36 line 2, page 37 line 13).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false

statements may jeopardize the validity of the application or any patent issued thereon.

By: John B. Classen
John B. Classen

7/6/94
Date

IPC:doh

Enclosures
Tables I-VI
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